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Most people agree that for a thorough understanding of any subject the mechanism of action must be known.

In the area of the biological sciences, particularly pathology, pharmacology, and medicine, the exact mechanism of action of the disease producing agent or of the drug which is used to cure the disease is not known. It can generally be said that we have a cause and effect understanding in this area, but we do not understand the actual biochemical changes which are being produced by either the disease producing agent or the drug or drugs which reverse the disease process.

We can go further and say that the exact mechanism of action of many materials which are essential for normal physiological function of the body are not known. For the above reasons these areas are generally taught as "classical" subjects and the presentation is essentially that of stating facts which have been gained from observation without a true explanation of the real mechanism of action.

Inroads are now being made into this area through research at the biochemical level and eventually a complete understanding of the mechanism of action of essential metabolites, disease production, and drug action will be achieved.

An idea of the type of work which must be done can be gained from study of the mechanism of action of the antibiotics. Because a considerable amount of work has been done with the antibiotics, it is possible to classify them basically into four groups as to their site of action on the microbial agent which they affect. These sites of action are:

1. On the bacterial cell wall
2. On the cell membrane
3. Somewhere within the chain of events which is responsible for protein synthesis
4. Somewhere within the nucleic acid metabolism scheme

To date, no antibiotics of therapeutic value have been found which interfere with intermediary metabolism. There may be very good reasons for this. If an antibiotic interfered with intermediary metabolism of the microbial agent, it would very possibly also interfere with the intermediary metabolism of the host and, therefore, be too toxic for general therapeutic use.

The only way an antibiotic which affects intermediary metabolism could be used for therapeutic purposes would be if it affected some intermediary metabolic process which is peculiar to the microbial agent. Such an agent has, in fact, been found though not among the antibiotics.

The sulfonamides act through such a mechanism of action. It was found that the sulfonamides act through competitive inhibition of para-amino-benzoic acid.[1] Basically, what happens is that due to the very close similarity in the structure of the active part of any sulfonamide and para-amino-benzoic acid, bacterial organisms will absorb the sulfonamide and "erroneously" substitute it for para-amino-benzoic acid. When this occurs, it prevents the reaction between pteric acid and glutamic acid which is necesary to form pteroyl-glutamic acid (another name for folic acid).

Unfortunately, the exact function of para-amino-benzoic acid in this reaction is not known. The sulfonamides do, however, block that vital role of para-amino-benzoic acid and bacterial multiplication ceases. The bacteria are not killed; their multiplication has only been stopped and in this case the body defense mechanisms

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must come into play to rid the body of disease.

There are two reasons why bacterial organisms are resistant to sulfonamides.

1. The microorganism produces its own para-amino-benzoic acid and does not depend on absorption of this metabolite from the media. It is not, therefore, affected by the sulfonamides.
2. The microbial organism does not produce its own folic acid but absorbs this from the surrounding media and, therefore, presents no place for the sulfonamide to act.

The reason that the sulfonamides do not pose any great threat to the host cell is that the host requires folic acid in its diet and does not produce its own. As a result, there is no intermediary metabolism site at which the sulfonamide can act. This mechanism of action also explains why cross reistance is not seen between sulfonamides and antibiotics to which a microbial agent would normally be susceptable.

The antibiotics have generally been placed into various groups according to their site of action as follows:

1. Those agents acting on the cell wall
   a. The penicillins
   b. The cephalosporins
   c. The bacitracins
   d. Cycloserine
   e. Vancomycin
   f. Ristocetin
   g. Novobiocin
   h. Oxamycin
2. Those agents acting on the cell membrane
   a. The polymyxins
   b. Colistin
   c. Novobiocin
   d. Tyrocidin
   e. Gramicidin
   f. Streptomycin
   g. Neomycin
   h. Kanamycin
   i. Nystatin
   j. Amphotericin
3. Those agents affecting protein synthesis
   a. The tetracyclines
   b. The streptomycins
   c. Neomycin
   d. Erythromycin
   e. Chloramphenicol
   f. Tylosin
   g. Lincomycin
   h. Oleandomycin
   i. Kanamycin
4. Those affecting nucleic acid metabolism
   a. Actinomycin
   b. Griseofulvin

It is very evident that some antibiotics act at more than one site or place in the bacterial agents vital metabolic processes. Another very outstanding defect in this breakdown of bacterial action is that it does not explain the exact mechanism of action which must be different for antibiotics within the same grouping since many times, cross resistance is not seen among antibiotics having the same general site of action. This implies that the specific site and the specific mechanism of action must be quite different.

Presumably, the specific site and mechanism of action of antibiotics which are closely related chemically is the same because as a general rule those antibiotics which are closely chemically related show complete cross resistance. The specific mechanism of action has been studied for very few antibiotics, however, and although the general site of action is known, the specific chemical site of action has not generally been determined even for these. A brief, somewhat simplified discussion of those mechanisms of actions of antibiotics which are best understood follows. At this point in the practice of Veterinary Medicine, the penicillins are the most important of the group of antibiotics which act on the bacterial cell wall. This also is the antibiotic whose mechanism of action has been worked out in the greatest detail.

Penicillin prevents formation of the cell wall of the bacterium by interfering with the formation of mucopeptides which are necessary in the cell wall.[2,3,4] The penicillins are generally considered to be bacteriocidal, actually killing bacteria, rather than bacteriostatic which simply inhibits growth. The evidence indicates that penicillin interferes with cell wall growth to cause its bacteriocidal effect. Specifically, penicillin blocks the formation of a mucopeptide polymer which, in staphylococci, consists of N-acetylmuramic

acid, N-acetylglucosamine, glycine, D-glutamic acid, L-lysine, D-alanine, and L-alanine. In the Gram + bacteria, mucopeptides form the most important and possibly only rigid supports of the cell. The rigid supports of the cell wall protect the underlying cell membrane from damage by the high internal osmotic pressure present when bacteria are growing in a media of normal osmolarity. This high pressure results from the concentration of solutes within the cell. The resulting damage to the unprotected cell membrane leads to death of the cell and many times even to lysis of the cell. What is actually happening to the bacterium is that the main mucopeptide chain of N-Ac-glucosamine and N-Ac-muramic acid continues to form but the linkages between the side changes are blocked.[5] What happens then is that the peptides which would normally be utilized to form these cross links accumulate in the cell and, as a result, attract water in an attempt to equalize the osmotic pressure. Since the cell wall is defective, it cannot give support and as the water goes into the cell, the cell membrane stretches until finally it bursts, and the bacterium is dead.[6,7,8] There is evidence that penicillin can also kill dormant bacteria, but thhe mechanism for this has not yet been worked out.

That Gram negative bacteria are less sensitive to penicillin is well known. Gram negative bacteria do undergo a swelling procedure when grown in the presence of penicillin, but they do not actually rupture. Penicillin acts only on the muramic acid containing mucopeptides which make up the main supporting structure of the gram positive bacteria. Gram negative bacteria also contain muramic acid mucopeptides but in addition have other supporting materials so that even if the mucopeptide support is lost they are still strong enough to resist rupture. It takes higher concentrations of penicillin to suppress gram negatives than gram positives but many can be suppressed with it.

On first examination it would appear that penicillin would also be detrimental to the animal cells. Penicillin, however, is very nontoxic to animal cells because it acts only on muramic acid containing mucopeptides, and these are found only in bacterial cell walls. The cell membrane of an animal does not contain muramic acid. Toxicity which is usually associated with penicillin is due to the form of the penicillin rather than to penicillin itself.

Of those agents which inferfere with protein synthesis, streptomycin has been studied to the greatest extent and lends itself to a fairly extensive discussion.

Streptomycin attaches to the 30S subunit of the ribosomes.[9,10] Exactly where or how it attaches is not known. Studies to date show that the streptomycin must attach to the 30S subunit before the two subunits of the ribosome combine to form the entire ribosome. The site on the 30S subunit is very specific, and those bacteria which do not have this site are resistant.[11,12,13,14] This site presumably is also absent on the mammalian ribosome. In those bacteria which are susceptable after the streptomycin attaches and the ribosome is formed, the ribosome starts to incorrectly incorporate the amino acid, phenylalanine, into proteins which are formed.[11,15] For example, pheylalanine may be substituted for isoleucine, leucine, serine, or some other amino acid which has a genetic code similar to phenylalanine. When this occurs, proteins are still formed but the proteins formed are nonfunctional and, as a result, all bacterial multiplication and activity stops.

Because of the way it acts, streptomycin is effective only in bacteria which are rapidly multiplying, and it has little or no effect on dormant bacteria. There is some evidence that streptomycin also affects the cell membrane, exerting a detergent-like effect, but its main activity is to interfere with protein synthesis. Another important consideration in the use of streptomycin is that it generally does not kill bacteria. Because of this, the body defense mechanisms must be functioning before satisfactory results are obtained. In other words, it is most effective in the acute stages of the infectious disease process.

Another group of antibiotics which fall into this same group as far as mechanism of action is concerned are the tetracyclines. The most common are oxytetracycline, chlortetracycline, tetracycline, and de-

methychlortetracycline. The exact mechanism of action of these is not completely understood, but three theories have been advanced to explain their action. They are:

1. They actively chelate cations.
2. They inhibit essential enzyme systems.
3. They suppress protein synthesis.

There is a tendency to discount the first theory, that the effect is due to chelation of ions. It is true that the tetracyclines do chelate certain cations, but it appears highly unlikely that this is their main action because extensive chelation of cations would produce deficiency symptoms in the animal, and this does not occur in tetracycline therapy.

This fact has a practical application in that absorption from the gastrointestinal tract is increased if these antibiotics are administered in the absence of calcium and magnesium. This needs to be especially taken into account when the tetracyclines are administered orally to animals on all milk diets.

The last two theories (inhibition of essential enzyme systems and suppression of protein synthesis) may in fact be one and the same. Enzymes are proteins, and if an antibiotic suppresses protein formation, it will suppress enzyme formation and, thereby, interfere with enzyme systems. It has definitely been shown that the tetracyclines suppress protein formation but exactly how is not known.[16] At least it has not been studied to the degree that streptomycin has.

Those antibiotics which have been described as having a detergent-like effect on the cell membrane present a rather vague picture when one tries to explain how they could possess a detergent- like effect which is detrimental to the point of causing bacteriostatic or even bacteriocidal activity. It would appear that these agents would affect the host cells in the same manner. Yet, in practice this presumably does not occur. This indicates that the conditions of the cell membranes must differ from the host cell to the bacterial cell if this mechanism and site of action is correct.

An excellent review of the information available on the mechanism of action of antibiotics has been published.[17] As of the date of that publication, the only antibiotic whose activity could be completely explained from a biochemical standpoint was D-cycloserine.

It seems logical to assume that if the exact chemical site of action and the exact chemical reaction at this site were known, it would be possible to administer chemical compounds to stop almost any disease process. Further, if we could administer such specific chemicals, it might be possible to avoid all of the toxic reactions which are now associated with antibiotic therapy.

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